Republic of Korea, [6]Asia-Pacific Center for Theoretical Physics, Pohang, Republic of Korea, [7]KAIST Institute Korea Advanced Institute of Science and Technology (KAIST), the BioCentury, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, Republic of Korea.
Inositol pyrophosphates such as IP_7 (5-diphosphoinositol pentakisphosphate) are highly energetic inositol metabolites containing pyrophosphate bonds. While IP_7 are known to regulate various biological events like metabolism, the molecular site of IP_7 action in vesicle trafficking remains elusive. Here, we showed that IP_7 potently inhibits synaptic vesicle exocytosis in both *invitro* reconstitution and cell culture settings. IP_7 was identified as a high-affinity ligand for synaptotagmin 1 (Syt1), a calcium sensor essential for membrane fusion. Interactions of IP_7 with Syt1 reduced the binding between Syt1 and Ca^{2+} as well as endowed Syt1 with its own negative effect, thereby suppressing Syt1 activation. These findings reveal a role of IP_7 as an inhibitor of the exocytotic pathway and expand our understanding of the signaling mechanisms of inositol pyrophosphates.

2552-Pos Board B244
C2B Domain in Synaptotagmin I Induces Membrane Bending Only After Conformational Change
Zhe Wu, Schulten Klaus.
University of Illinois Urbana Champaign, Urbana, IL, USA.
Neuronal exocytosis is mediated by a Ca^{2+}-triggered membrane fusion event that joins synaptic vesicles and presynaptic membrane. In this event, synaptotagmin I plays a key role as a Ca^{2+} sensor protein that binds to and bends the presynaptic membrane with its C2B domain and, thereby, triggers membrane fusion. We report free energy calculations according to which C2B-induced membrane bending is preceded by a Ca^{2+}- and membrane-dependent conformational transition, in which C2B attaches to the membrane, moves its C-terminal helix from the orientation seen in the membrane-free crystal/NMR structures as pointing away from the membrane (helix up), to an orientation pointing towards the membrane (helix down). In the C2B ''helix down'' state, lipid tails in the proximal membrane bilayer leaflet interact with the moved helix and become disordered, while tails in the distal leaflet, to keep in contact with the proximal leaflet, become stretched and ordered. The difference in lipid tail packing between the two leaflets results in an imbalance of pressure across the membrane and, thereby, causes membrane bending. The lipid disordering in the proximal membrane leaflet should facilitate Ca^{2+}-triggered membrane fusion.

2553-Pos Board B245
Mitofusin Proteins Tether Proteoliposomes as Shown by Cryo-EM
Jeanne Morin-Leisk, Jenny Hinshaw.
National Institutes of Health, Bethesda, MD, USA.
A balance between mitochondrial membrane fusion and fission is required for normal mitochondrial morphology and function. Shifts in mitochondrial morphology as a result of changes in the balance of membrane fusion and fission accompany cellular differentiation and changes in metabolic state. It is thought that the severe, early-onset peripheral neuropathy Charcot-Marie-Tooth type 2A disease is a manifestation of mitochondrial fusion malfunction as a result of a mutation of one of the two human mitofusins, mitofusin 2. Our aim is to reveal the mechanism by which mitofusins cause membrane fusion. Both mammalian mitofusins contain a large GTPase domain at their N-terminus followed by two transmembrane domains and a short C-terminal domain. It is predicted that GTP hydrolysis and mitofusin conformational changes are coupled to membrane fusion. However, the precise mechanism for mitofusin-mediated membrane fusion remains an open question. In fact, it is still unknown if mitochondrial outer membrane fusion proceeds through the canonical steps of tethering, docking, fusion, and disassembly. To gain insight into the conformational changes that lead to membrane fusion we are examining the structure of mitofusins in a lipid bilayer. As two-pass transmembrane proteins, mitofusins have proven difficult to express and purify in their full-length state and are poor candidates for crystallography. Here we present a purification strategy and the first cryo-em images of full-length mitofusins in a lipid bilayer. In addition, we show that mitofusins tether proteolipsosomes by forming oligomers that interact in trans between synthetic membranes. This implies that mitofusins are sufficient for driving the first step of membrane fusion.

2554-Pos Board B246
Role of Model Proteins on Membrane Fusion
Hemant K. Kashyap, Michael L. Klein, Giacomo Fiorin.
Institute for Computational Molecular Science, Temple University, Philadelphia, PA, USA.
Within neural cells, synaptic vesicles carry neurotransmitters (signaling molecule) through the plasma membrane. In order to do so synaptic vesicles must fuse with the plasma membrane, so that the enclosed neurotransmitter can cross it. Typically, without fusion proteins membrane fusion occurs on a very long time scale. However, it has been established that the fusion occur via fusion proteins (FPs), which initially are bound to one or both of the fusing membranes via a trans-membrane (TM) helix, utilize energetically favorable conformational transitions to lower the activation energy for membrane fusion, and thus are key participants in shaping the energy landscape by facilitating bilayer-bilayer apposition and dehydration as bilayers come into more intimate contact. It is also widely accepted that the trans-membrane (TM) part of the FPs has vital role in governing the fusion, fusion does not occur if TM is replaced with lipid molecules. In order to understand how the TM segment of FPs facilitates membrane fusion, we used a model protein that mimics mostly the TM part of the FPs, of similar dimensions to an α-helix. In doing so, the length of the model protein was chosen to match the thickness of a POPC bilayer. To retain the trans-membrane orientation of the model proteins without adding the soluble domains, we truncated the ends with polar groups. The middle portion was kept non-polar. We observed via coarse-grained molecular dynamics simulations that the self-aggregation of the model proteins greatly enhances the rate of formation of hemi-fused intermediate states. Also, the model-proteins if present in relatively higher concentration drive the attachment of a 20nm size vesicle to a flat bilayer.

2555-Pos Board B247
Waiting Times for Fusion Depend on the Number of Snares at the Fusion Site
Hakhamanesh Mostafavi[1], Benjamin S. Stratton[1], Jason M. Warner[1], Erdem Karatekin[2], Ben O'Shaughnessy[1].
[1]Chemical Engineering, Columbia University, New York, NY, USA, [2]Department of Cellular and Molecular Physiology, Yale University, New Haven, CT, USA.
SNARE proteins mediate membrane fusion in fundamental cellular processes such as exocytosis. Formation of SNAREpin complexes, from vesicle associated v-SNAREs and target-membrane associated t-SNAREs, is thought to trigger fusion. However, the detailed mechanisms remain unclear and widely varying SNARE requirements for fusion are reported, 2-15 in vivo and 1-11 in vitro. Here we mathematically modeled the collective behavior of SNAREpins at the fusion site. The SNAREpins organize into a ring, as indicated by experiment, and steric interactions between SNAREpins expand the ring and pull membranes together, while the membranes repel one another through hydration and electrostatic forces. The net effect is a high pressure at the intermembrane contact point, sufficient to fuse the membranes should it exceed the measured pressure threshold for fusion [Wong et al., Biophys J, 1999]. Monte Carlo simulations showed that this pressure fluctuates considerably: with fewer SNAREpins, the mean pressure was lower, the probability of super-fusion pressure sharply reduced, and the waiting time for fusion increased. Thus, consistent with experiment, any number of SNAREpins can drive fusion, but the waiting time is strongly dependent on that number. For example, for a typical membrane composition, fast fusion of 50nm vesicles is predicted to require 11 SNAREpins, but 4 SNAREpins also achieve fusion ~50 times more slowly. We also find that waiting times increase for larger vesicles and for longer SNARE linker domains, in qualitative agreement with studies reporting lower fusion activity with elongated linkers. Our results suggest that fully zippered SNAREs work in concert to trigger fusion, by generating high local pressures that destabilize membranes into fusion pores. This naturally explains the widely reported variations in SNARE requirements for fusion.

2556-Pos Board B248
Field Theoretic Approach for the Energetics of Stalk Formation
Rolf Ryham[1], Thomas Klotz[1], Lihan Yao[1], Fredric S. Cohen[2].
[1]Mathematics, Fordham University, Bronx, NY, USA, [2]Molecular Biophysics and Physiology, Rush University, Chicago, IL, USA.
A numerical procedure was developed to calculate the minimum energy pathway for stalk formation between two flat and parallel lipid bilayer membranes. Bilayers were modeled as axially symmetric, freely deformable surfaces with lipid director fields. Energies were obtained by calculating the elastic energy–splay, tilt, and stretching–of each monolayer, along with undulation and hydration forces that repel membranes from each other. The topological transition between the planar and stalk phase was explicitly treated by allowing the bilayer to form a circular fissure along the axis of symmetry; the energy of fissure was approximated by the water-hydrocarbon surface and the area of exposed acyl chains. Generally, stalk formation is not spontaneous; it requires that external energy, such as provided by fusion proteins, be supplied to the bilayers. To quantify the energy required to surmount the

transition barrier that separates a stalk and the original separate membranes, least energy paths were calculated by the string method. The string method finds a morphological transition between two energy basins_in this case the planar and stalk phases_that minimizes the height of the energy barrier. Sequences of images of bilayer shapes calculated by the string method show that opposing bilayers deform into cusped shapes that then form hydrophobic fissures in each membrane, which then promotes merger into a stalk shape. The energetics of stalk formation and morphologies of stalks were analyzed as a function of lipid composition and bilayer dimensions. The shapes of stalks were compared with shapes of stalks that form between stacks of planar bilayers, as obtained by x-ray diffraction: the theoretically calculated shapes are in agreement with those measured experimentally. Supported by NIH R01 GM101539.

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Variable and Concerted Cooperativity in Snare-Mediated Memebrane Fusion

Alex Kreutzberger[1], Javier M. Hernandez[2], Volker Kiessling[1], Lukas K. Tamm[1], Reinhard Jahn[2].
[1]University of Virginia, Charlottesville, VA, USA, [2]Max Planck Institute for Biophysical Chemistry, Göttingen, Germany.

The soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex drives the majority of intracellular and exocytic membrane fusion events. If and how SNAREs cooperate to form a single fusion pore has been a subject of intense study with estimates ranging from a single SNARE complex to fifteen being necessary for fusion. Here we show that there is likely no universally conserved number of complexes involved and that this number varies depending on membrane properties. In particular, we found that the threshold for efficient fusion depends on membrane curvature. When docking rates of small (~40 nm) and large (~100 nm) liposomes reconstituted with different synaptobrevin (the SNARE present in synaptic vesicles) densities are taken into account, the fusion efficiency of large SNARE-liposomes declined even when there were more than ~23-30 synaptobrevins present on the entire liposome. The conclusion derived from ensemble measurements that membrane curvature modulates the number of complexes required for fusion was further confirmed by experiments analyzing fusion of single vesicles to planar supported bilayers. We propose that the local number of SNARE complexes required at the site of fusion depends on the energy barrier of a particular fusion reaction and that the complexes assemble in a concerted (non-sequential) fashion for efficient execution of fusion.

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The Snare Motif of Membrane-Anchored Synaptobrevin Exhibits an Aqueous-Interfacial Partitioning that is Modulated by Membrane Curvature

Binyong Liang[1], Damian Dawidowski[2], Jeffrey F. Ellena[3], Lukas K. Tamm[1], David S. Cafiso[2].
[1]Department of Molecular Physiology and Biological Physics, University of Virginia, Charlottesville, VA, USA, [2]Department of Chemistry, University of Virginia, Charlottesville, VA, USA, [3]Biomolecular Magnetic Resonance Research Core, University of Virginia, Charlottesville, VA, USA.

The SNARE forming motif of the vesicle associated protein synaptobrevin 2, is generally thought to be unstructured in the aqueous phase prior to assembly of the neuronal core SNARE complex. Here, the structure and interfacial association of the full-length vesicle SNARE, synaptobrevin, was compared in four different lipid environments using NMR and EPR spectroscopy. In micelles, segments of the SNARE motif are helical and associated with the interface. However, the fraction of helix and interfacial association decreases as synaptobrevin is moved from micelle to bicelle to bilayer environments, suggesting that the tendency towards interfacial association is sensitive to membrane curvature. In bilayers, the SNARE motif of synaptobrevin transiently associates with the lipid interface, and regions that are helical in micelles are in conformational and environmental exchange in bicelles and bilayers. This work demonstrates that the SNARE motif of synaptobrevin has a significant propensity to form a helix and exchange with the membrane interface prior to SNARE assembly. This transient interfacial association and its sensitivity to membrane curvature and/or defects likely play a role in SNARE recognition events that drive membrane fusion.

2559-Pos Board B251

Pre-Fusion Structure of Syntaxin 1A Suggests Pathway for Folding into Neuronal Trans-Snare Complex Fusion Intermediate

Volker Kiessling, Binyong Liang, Lukas K. Tamm.
Physiology, University of Virginia, Charlottesville, VA, USA.

Assembly of the three neuronal SNARE proteins synaptobrevin-2, syntaxin 1A, and SNAP-25 is the key step that leads to exocytotic fusion of synaptic vesicles. In the fully assembled SNARE complex, these three proteins form a coiled-coil four-helix bundle structure by interaction of their respective SNARE motifs. Although biochemical and mutational analyses strongly suggest that the heptad-repeat SNARE motifs zipper into the final structure, little is known about the pre-fusion state of individual membrane-bound SNAREs and how they change conformation from the unzippered pre-fusion to the zippered post-fusion state in a membrane environment. We have solved the solution NMR structure of micelle-bound syntaxin 1A in its pre-fusion conformation. In addition to the transmembrane helix, the SNARE motif consists of two well-ordered, membrane-bound helices separated by the "0-layer" residue Gln226. This unexpected structural order of the N- and C-terminal halves of the uncomplexed SNARE motif suggests the formation of partially zippered SNARE complex intermediates with the "0-layer" serving as a proof-reading site for correct SNARE assembly. Interferometric fluorescence measurements in lipid bilayers confirm that the open SNARE motif helices of syntaxin interact with lipid bilayers and that association with the other target-membrane SNARE SNAP-25 lifts the SNARE motif off the membrane as a critical prerequisite for SNARE complex assembly and membrane fusion.

2560-Pos Board B252

Complexin-1 Enhances the On-Rate of Vesicle Docking via Simultaneous Snare and Membrane Interactions

Jiajie Diao, Daniel J. Cipriano, Axel T. Brunger.
Stanford and HHMI, Stanford, CA, USA.

In synaptic terminals, complexin is thought to have inhibitory and activating roles for spontaneous mini-release and evoked synchronized neurotransmitter release, respectively. We used single vesicle-vesicle microscopy imaging to study the effect of complexin-1 on the docking on-rate between vesicles that mimic synaptic vesicles and vesicles that mimic the plasma membrane. We found that complexin-1 enhances the on-rate of docking between synaptic vesicle mimics containing full-length synaptobrevin-2 and full-length synaptotagmin-1 and plasma membrane mimicking vesicles containing full-length syntaxin-1A and SNAP-25A. This effect requires the C-terminal domain of complexin-1 which binds to the membrane, the presence of PS in the membrane, and the core region of complexin-1 which binds to the SNARE complex.

References

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2561-Pos Board B253

Quantitative Molecular Modeling of Membrane Curvature Induction by an Amphipathic Helix

Alexander J. Sodt, Richard W. Pastor.
NHLBI/NIH, National Institutes of Health, Bethesda, MD, USA.

The cell must create membrane curvature in vesicle formation processes like clathrin-mediated endocytosis and SNARE-mediated exocytosis.
Two mechanisms of curvature induction have been studied: Scaffolding, in which a curved protein complex enforces its own shape on the membrane, and hydrophobic insertion, in which peptide material inserts directly into the bilayer and modifies the surface properties.
This work studies the latter.
A detailed molecular model (the CHARMM forcefield) of an amphipathic helix embedded at the surface of the lipid membrane is shown to create a substantial curvature preference.
The model is directly contrasted with the prediction of weaker induction by a continuum elastic treatment.
The discrepancy is analyzed in terms of how curvature is influenced by lipid/inclusion shape and specific chemical interactions.